

**AMENDMENTS TO THE CLAIMS**

1. (withdrawn) A method for real-time detecting and quantifying a nucleic acid template in a PCR mixture comprising the steps of
  - a) thermally cycling the PCR mixture, wherein the PCR mixture comprises a thermostable polymerase, the nucleic acid template, primers to amplify at least one amplicon from the nucleic acid template, and a double stranded DNA dye, wherein the amplicon has a melting temperature of  $T_m$ ;
  - b) obtaining cycle by cycle a pre- $T_m$  emission at a MT below the  $T_m$  and a post- $T_m$  emission at the a MT above the  $T_m$ ;
  - c) determining cycle by cycle an emission amount of the amplicon, which is the difference between the pre- $T_m$  emission and the post- $T_m$  emission.
2. (withdrawn) The method of claim 1 wherein the double stranded DNA dye is a double stranded DNA intercalating dye.
3. (withdrawn) The method of claim 2 wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.
4. (withdrawn) The method of claim 1 wherein the double stranded DNA dye is a primer-based double stranded DNA dye.
5. (withdrawn) The method of claims 4 wherein the primer-based double stranded DNA dye is selected from the group consisting of fluorescein, FAM, JOE, HEX, TET, Alexa Fluor 594, ROX, and TAMRA, rhodamine, BODIPY-FL.

6. (withdrawn) The method of claim 1 wherein the MT below the  $T_m$  is 0.25 °C below, 0.5 °C below, 1.0 °C below, 1.5 °C below, or 2.0 °C below the  $T_m$ .
7. (withdrawn) The method of claim 1 wherein the MT above the  $T_m$  is 0.25 °C above, 0.5 °C above, 1.0 °C above, 1.5 °C above, or 2.0 °C above the  $T_m$ .
8. (withdrawn) The method of claim 1 wherein the emission amount of the amplicon is obtained through a computer program which performs a calculation of subtracting the pre- $T_m$  emission from the post- $T_m$  emission or the post- $T_m$  emission from the pre- $T_m$  emission.
9. (original) A method for real-time detecting and quantifying a first nucleic acid template and a second nucleic acid template in a PCR mixture comprising the steps of
  - a) thermally cycling a PCR mixture wherein the PCR mixture comprises a thermostable polymerase, a double stranded DNA dye, the first template and the second template, primers for amplifying a first amplicon from the first template and a second amplicon from the second template, and wherein the first amplicon has a first  $T_m$  and the second amplicon has a second  $T_m$  and the first  $T_m$  is less than the second  $T_m$ ;
  - b) obtaining cycle by cycle a first emission at a first MT between an annealing/extension temperature and the first  $T_m$  and a second emission at a second MT between the first  $T_m$  and the second  $T_m$ ;
  - c) determining cycle by cycle a first emission amount of the first amplicon which is the difference between the first emission and the second emission, and a second emission amount of the second amplicon which is the second emission.

10. (original) The method of claim 9 further comprising a step of obtaining cycle by cycle a third emission at a third MT between the second  $T_m$  and a total denaturing temperature, wherein the second emission amount is the difference between the second emission and the third emission.

11. (original) The method of claim 9 wherein the double stranded DNA dye is a double stranded DNA intercalating dye.

12. (original) The method of claim 11 wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

13. (original) The method of claim 9 wherein the double stranded DNA dye is a primer-based double stranded DNA dye.

14. (original) The method of claims 13 wherein the primer-based double stranded DNA dye is selected from the group consisting of fluorescein, FAM, JOE, HEX, TET, Alexa Fluor 594, ROX, and TAMRA, rhodamine, BODIPY-FL.

15. (original) The method of claim 9 wherein the first MT is 0.25 °C below the first  $T_m$ , 0.5 °C below the first  $T_m$ , 1.0 °C below the first  $T_m$ , 1.5 °C below the first  $T_m$ , or 2.0 °C below the first  $T_m$ , and wherein the first MT is higher than the annealing temperature.

16. (original) The method of claim 9 wherein the second MT is 0.25 °C below the second  $T_m$ , 0.5 °C below the second  $T_m$ , 1.0 °C below the second  $T_m$ , 1.5 °C below the second  $T_m$ , or 2.0 °C below the second  $T_m$ , and wherein the second MT is higher than the first  $T_m$ .

17. (original) The method of claim 9 wherein the second MT is 0.25 °C above the first  $T_m$ , 0.5 °C above the first  $T_m$ , 1.0 °C above the first  $T_m$ , 1.5 °C above the first  $T_m$ , or 2.0 °C above the first  $T_m$ , and wherein the second MT is less than the second  $T_m$ .

18. (original) The method of claim 9 wherein the second MT is the first  $T_m + 0.25^{\circ}\text{C} < \text{the second MT} < \text{the second } T_m - 0.25^{\circ}\text{C}$ , the first  $T_m + 0.5^{\circ}\text{C} < \text{the second MT} < \text{the second } T_m - 0.5^{\circ}\text{C}$ , the first  $T_m + 1.0^{\circ}\text{C} < \text{the second MT} < \text{the second } T_m - 1.0^{\circ}\text{C}$ , the first  $T_m + 1.5^{\circ}\text{C} < \text{the second MT} < \text{the second } T_m - 1.5^{\circ}\text{C}$ , or the first  $T_m + 2.0^{\circ}\text{C} < \text{the second MT} < \text{the second } T_m - 2.0^{\circ}\text{C}$ .

19. (original) The method of claim 10 wherein the third MT is 0.25 °C above the second  $T_m$ , 0.5 °C the second  $T_m$ , 1.0 °C above the second  $T_m$ , 1.5 °C above the second  $T_m$ , or 2.0 °C above the second  $T_m$ , and wherein the third MT is less than the total denaturing temperature.

20. (original) The method of claim 9 wherein the emission amount of the first amplicon is obtained through a computer program performing a calculation of subtracting the first emission from the second emission or subtracting the second emission from the first emission.

21. (original) A method for real-time detecting and quantifying a first nucleic acid template and a second nucleic acid template in a PCR mixture comprising the steps of:

- a) thermally cycling a PCR mixture wherein the PCR mixture comprises a thermostable polymerase, a double stranded DNA dye, the first template and the second template, primers for amplifying a first amplicon from the first template and a second amplicon from the second template, and wherein the first amplicon has a first  $T_m$  and the

second amplicon has a second  $T_m$  and the first  $T_m$  is less than the second  $T_m$ ;

- b) obtaining cycle by cycle a first pre- $T_m$  emission at a MT below the first  $T_m$  and a first post- $T_m$  emission at the a MT above the first  $T_m$  and a second pre- $T_m$  emission at a MT below the second  $T_m$  and a second post- $T_m$  emission at the a MT above the second  $T_m$ ;
- c) determining cycle by cycle a first emission amount of the first amplicon which is the difference between the first pre- $T_m$  emission and the first post- $T_m$  emission; and a second emission amount of the second amplicon which is the difference between the second pre- $T_m$  emission and the second post- $T_m$  emission.

22. (original) The method of claim 21 wherein the double stranded DNA dye is a double stranded DNA intercalating dye

23. (original) The method of claim 22 wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

24. (original) The method of claim 21 wherein the double stranded DNA dye is a primer-based double stranded DNA dye.

25. (original) The method of claims 24 wherein the primer-based double stranded DNA dye is selected from the group consisting of fluorescein, FAM, JOE, HEX, TET, Alexa Fluor 594, ROX, and TAMRA, rhodamine, BODIPY-FL.

26. (original) The method of claim 21 wherein the MT below the first  $T_m$  and/or the second  $T_m$  are 0.25 °C below, 0.5 °C below, 1.0 °C below, 1.5 °C below, or 2.0 °C below.

27. (original) The method of claim 21 wherein the  $T_m$  above the first  $T_m$  and/or the second  $T_m$  are  $0.25^\circ\text{C}$  above,  $0.5^\circ\text{C}$  above,  $1.0^\circ\text{C}$  above,  $1.5^\circ\text{C}$  above, or  $2.0^\circ\text{C}$  above.

28. (original) The method of claim 21 wherein the emission amount of the amplicons is obtained through a computer program performing the calculation of subtracting the pre- $T_m$  emission from the post- $T_m$  emission or subtracting the post- $T_m$  emission from the pre- $T_m$  emission.

29. (withdrawn) A method for real-time detecting and quantifying a total of  $n$  nucleic acid templates in a PCR mixture comprising the steps of:

a) thermally cycling a PCR mixture, wherein the PCR mixture comprises a thermostable polymerase, nucleic acid templates including  $n$  nucleic acid templates, primers for amplifying  $n$  amplicons, and a double stranded DNA dye;

b) obtaining cycle by cycle a  $MT_k$  emission at  $MT_k$  and  $MT_{(k+1)}$ , wherein  $T_{m(k-1)} < MT_k < T_{mk} < MT_{(k+1)} < T_{m(k+1)}$ ,  $T_{mk}$  is the  $T_m$  of a  $k$ th amplicon,  $T_{m(k-1)}$  is the  $T_m$  of a  $(k-1)$ th amplicon except that  $T_{m(k-1)}$  is an annealing and/or an extension temperature when  $k=1$ ,  $T_{m(k+1)}$  is the  $T_m$  of a  $(k+1)$ th amplicon except that  $T_{m(n+1)}$  is a total denaturing temperature when  $k=n$ , and  $k$  and  $n$  are positive integers,  $1 \leq k \leq n$ , and  $n \geq 2$ ;

c) determining cycle by cycle an emission amount of the  $k$ th amplicon which is the difference between the  $MT_k$  emission and the  $MT_{(k+1)}$  emission.

30. (withdrawn) The method of claim 29 wherein the double stranded DNA dye is a double stranded DNA intercalating dye.

31. (withdrawn) The method of claim 30 wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

32. (withdrawn) The method of claim 29 wherein the double stranded DNA dye is a primer-based double stranded DNA dye that is covalently linked to the primers.

33. (withdrawn) The method of claims 32 wherein the primer-based double stranded DNA dye is selected from the group consisting of fluorescein, FAM, JOE, HEX, TET, Alexa Fluor 594, ROX, and TAMRA, rhodamine, BODIPY-FL.

34. (withdrawn) The method of claim 29 wherein  $T_{m(k-1)} + 0.25^{\circ}\text{C} < MT_k < T_{mk}$ ,  $T_{m(k-1)} + 0.5^{\circ}\text{C} < MT_k < T_{mk}$ ,  $T_{m(k-1)} + 1.0^{\circ}\text{C} < MT_k < T_{mk}$ ,  $T_{m(k-1)} + 1.5^{\circ}\text{C} < MT_k < T_{mk}$ , or  $T_{m(k-1)} + 2.0^{\circ}\text{C} < MT_k < T_{mk}$ .

35. (withdrawn) The method of claim 29 wherein  $T_{mk} + 0.25^{\circ}\text{C} < MT_{(k+1)} < T_{m(k+1)}$ ,  $T_{mk} + 0.5^{\circ}\text{C} < MT_{(k+1)} < T_{m(k+1)}$ ,  $T_{mk} + 1.0^{\circ}\text{C} < MT_{(k+1)} < T_{m(k+1)}$ ,  $T_{mk} + 1.5^{\circ}\text{C} < MT_{(k+1)} < T_{m(k+1)}$ , or  $T_{mk} + 2.0^{\circ}\text{C} < MT_{(k+1)} < T_{m(k+1)}$ .

36. (withdrawn) The method of claim 29 wherein  $T_{m(k-1)} < MT_k < T_{mk} - 0.25^{\circ}\text{C}$ ,  $T_{m(k-1)} < MT_k < T_{mk} - 0.5^{\circ}\text{C}$ ,  $T_{m(k-1)} < MT_k < T_{mk} - 1.0^{\circ}\text{C}$ ,  $T_{m(k-1)} < MT_k < T_{mk} - 1.5^{\circ}\text{C}$ , or  $T_{m(k-1)} < MT_k < T_{mk} - 2.0^{\circ}\text{C}$ .

37. (withdrawn) The method of claim 29 wherein  $T_{mk} < MT_{(k+1)} < T_{m(k+1)} - 0.25^{\circ}\text{C}$ ,  $T_{mk} < MT_{(k+1)} < T_{m(k+1)} - 0.5^{\circ}\text{C}$ ,  $T_{mk} < MT_{(k+1)} < T_{m(k+1)} - 1.0^{\circ}\text{C}$ ,  $T_{mk} < MT_{(k+1)} < T_{m(k+1)} - 1.5^{\circ}\text{C}$ , or  $T_{mk} < MT_{(k+1)} < T_{m(k+1)} - 2.0^{\circ}\text{C}$ .

38. (withdrawn) The method of claim 29 wherein  $T_{m(k-1)} + 0.25^{\circ}\text{C} < MT_k < T_{mk} - 0.25^{\circ}\text{C}$ ,  $T_{m(k-1)} + 0.5^{\circ}\text{C} < MT_k < T_{mk} - 0.5^{\circ}\text{C}$ ,  $T_{m(k-1)} + 1.0^{\circ}\text{C} < MT_k < T_{mk} - 1.0^{\circ}\text{C}$ ,  $T_{m(k-1)} + 1.5^{\circ}\text{C} < MT_k < T_{mk} - 1.5^{\circ}\text{C}$  or  $T_{m(k-1)} + 2.0^{\circ}\text{C} < MT_k < T_{mk} - 2.0^{\circ}\text{C}$ .

39. (withdrawn) The method of claim 29 wherein  $T_{mk} + 0.25^{\circ}\text{C} < MT_{(k+1)} < T_{m(k+1)} - 0.25^{\circ}\text{C}$ ,  $T_{mk} + 0.5^{\circ}\text{C} < MT_{(k+1)} < T_{m(k+1)} - 0.5^{\circ}\text{C}$ ,  $T_{mk} + 1.0^{\circ}\text{C} < MT_{(k+1)} < T_{m(k+1)} - 1.0^{\circ}\text{C}$ ,  $T_{mk} + 1.5^{\circ}\text{C} < MT_{(k+1)} < T_{m(k+1)} - 1.5^{\circ}\text{C}$ , or  $T_{mk} + 2.0^{\circ}\text{C} < MT_{(k+1)} < T_{m(k+1)} - 2.0^{\circ}\text{C}$ .
40. (withdrawn) The method of claim 29 wherein  $2 \leq n \leq 35$ ,  $2 \leq n \leq 18$ ,  $2 \leq n \leq 10$ ,  $2 \leq n \leq 7$ , or  $2 \leq n \leq 5$ .
41. (withdrawn) The method of claim 40 wherein  $n = 2, 3, 4$ , or  $5$ .
42. (withdrawn) The method of claim 29 wherein the PCR mixture further comprises a FRET based probe.
43. (withdrawn) The method of claim 42 wherein the FRET based probe is selected from the group consisting of a Taqman probe, a double-dye oligonucleotide probe, an Eclipse probe, a Molecular Beacon probe, a Scorpion probe, a Hybridization probe, a ResonSense probe, a Light-up probe, and a Hy-Beacon probe.
44. (withdrawn) The method of claim 29 wherein the PCR mixture further comprises a second primer-based double stranded DNA dye that emits differently from the double stranded DNA dye.
45. (withdrawn) The method of claim 29 wherein the emission amount of the  $k$ th amplicon is obtained through a computer program performing the subtraction of  $MT_k$  emission from  $MT_{(k+1)}$  emission or the subtraction of the  $MT_{(k+1)}$  emission from  $MT_k$  emission.
46. (withdrawn) A method for detecting and quantifying a total of  $n$  nucleic acid templates in multiplex real-time PCR comprising the steps of:

a) thermally cycling a PCR mixture, wherein the PCR mixture comprises a thermostable polymerase, nucleic acid templates including  $n$  nucleic acid templates, primers for amplifying  $n$  amplicons, and a double stranded DNA dye;

b) obtaining cycle by cycle a pre- $T_{mk}$  emission of the  $k$ th amplicon at a MT between  $T_{m(k-1)}$  and  $T_{mk}$  and a post- $T_{mk}$  emission of the  $k$ th amplicon at a MT between  $T_{mk}$  and  $T_{m(k+1)}$ , wherein  $T_{m(k-1)} < T_{mk} < T_{m(k+1)}$ ,  $T_{mk}$  is the  $T_m$  of a  $k$ th amplicon,  $T_{m(k-1)}$  is the  $T_m$  of a  $(k-1)$ th amplicon except that  $T_{m(k-1)}$  is an annealing and/or an extension temperature when  $k=1$ ,  $T_{m(k+1)}$  is the  $T_m$  of a  $(k+1)$ th amplicon except that  $T_{m(n+1)}$  is a total denaturing temperature when  $k=n$ , and  $k$  and  $n$  are positive integers,  $1 \leq k$ , and  $n \geq 2$ ;

c) determining cycle by cycle an emission amount of the  $k$ th amplicon which is the difference between the pre- $T_{mk}$  emission and the post- $T_{mk}$  emission.

47. (withdrawn) The method of claim 46 wherein the double stranded DNA dye is a double stranded DNA intercalating dye.

48. (withdrawn) The method of claim 47 wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

49. (withdrawn) The method of claim 46 wherein the double stranded DNA dye is a primer-based double stranded DNA dye.

50. (withdrawn) The method of claims 49 wherein the primer-based double stranded DNA dye is selected from the group consisting of fluorescein, FAM, JOE, HEX, TET, Alexa Fluor 594, ROX, and TAMRA, rhodamine, BODIPY-FI.

51. (withdrawn) The method of claim 46 wherein the MT between  $T_{m(k-1)}$  and  $T_{mk}$  is  $T_{m(k-1)} + 0.25^{\circ}\text{C} < \text{the MT between } T_{m(k-1)} \text{ and } T_{mk} < T_{mk}$ ,  $T_{m(k-1)} + 0.5^{\circ}\text{C} < \text{the MT between } T_{m(k-1)} \text{ and } T_{mk} < T_{mk}$ ,  $T_{m(k-1)} + 1.0^{\circ}\text{C} < \text{the MT between } T_{m(k-1)} \text{ and } T_{mk} < T_{mk}$ ,  $T_{m(k-1)} + 1.5^{\circ}\text{C} < \text{the MT between } T_{m(k-1)} \text{ and } T_{mk} < T_{mk}$ , or  $T_{m(k-1)} + 2.0^{\circ}\text{C} < \text{the MT between } T_{m(k-1)} \text{ and } T_{mk} < T_{mk}$ .

52. (withdrawn) The method of claim 46 wherein the MT between  $T_{mk}$  and  $T_{m(k+1)}$  is  $T_{mk} + 0.25^{\circ}\text{C} < \text{the MT between } T_{mk} \text{ and } T_{m(k+1)} < T_{m(k+1)}$ ,  $T_{mk} + 0.5^{\circ}\text{C} < \text{the MT between } T_{mk} \text{ and } T_{m(k+1)} < T_{m(k+1)}$ ,  $T_{mk} + 1.0^{\circ}\text{C} < \text{the MT between } T_{mk} \text{ and } T_{m(k+1)} < T_{m(k+1)}$ ,  $T_{mk} + 1.5^{\circ}\text{C} < \text{the MT between } T_{mk} \text{ and } T_{m(k+1)} < T_{m(k+1)}$ ,  $T_{mk} + 2.0^{\circ}\text{C} < \text{the MT between } T_{mk} \text{ and } T_{m(k+1)} < T_{m(k+1)}$ .

53. (withdrawn) The method of claim 46 wherein the MT between  $T_{m(k-1)}$  and  $T_{mk}$  is  $T_{m(k-1)} < \text{the MT between } T_{m(k-1)} \text{ and } T_{mk} < T_{mk} - 0.25^{\circ}\text{C}$ ,  $T_{m(k-1)} < \text{the MT between } T_{m(k-1)} \text{ and } T_{mk} < T_{mk} - 0.5^{\circ}\text{C}$ ,  $T_{m(k-1)} < \text{the MT between } T_{m(k-1)} \text{ and } T_{mk} < T_{mk} - 1.0^{\circ}\text{C}$ ,  $T_{m(k-1)} < \text{the MT between } T_{m(k-1)} \text{ and } T_{mk} < T_{mk} - 1.5^{\circ}\text{C}$ , or  $T_{m(k-1)} < \text{the MT between } T_{m(k-1)} \text{ and } T_{mk} < T_{mk} - 2.0^{\circ}\text{C}$ .

54. (withdrawn) The method of claim 46 wherein the MT between  $T_{mk}$  and  $T_{m(k+1)}$  is  $T_{mk} < \text{the MT between } T_{mk} \text{ and } T_{m(k+1)} < T_{m(k+1)} - 0.25^{\circ}\text{C}$ ,  $T_{mk} < \text{the MT between } T_{mk} \text{ and } T_{m(k+1)} < T_{m(k+1)} - 0.5^{\circ}\text{C}$ ,  $T_{mk} < \text{the MT between } T_{mk} \text{ and } T_{m(k+1)} < T_{m(k+1)} - 1.0^{\circ}\text{C}$ ,  $T_{mk} < \text{the MT between } T_{mk} \text{ and } T_{m(k+1)} < T_{m(k+1)} - 1.5^{\circ}\text{C}$ , or  $T_{mk} < \text{the MT between } T_{mk} \text{ and } T_{m(k+1)} < T_{m(k+1)} - 2.0^{\circ}\text{C}$ .

55. (withdrawn) The method of claim 46 wherein the MT between  $T_{m(k-1)}$  and  $T_{mk}$  is  $T_{m(k-1)} + 0.25^{\circ}\text{C} < \text{the MT between } T_{m(k-1)} \text{ and } T_{mk} < T_{mk} - 0.25^{\circ}\text{C}$ ,  $T_{m(k-1)} + 0.5^{\circ}\text{C} < \text{the MT between } T_{m(k-1)} \text{ and } T_{mk} < T_{mk} - 0.5^{\circ}\text{C}$ ,  $T_{m(k-1)} + 1.0^{\circ}\text{C} < \text{the MT between } T_{m(k-1)} \text{ and } T_{mk} < T_{mk} - 1.0^{\circ}\text{C}$ ,  $T_{m(k-1)} + 1.5^{\circ}\text{C} < \text{the MT between } T_{m(k-1)} \text{ and } T_{mk} < T_{mk} - 1.5^{\circ}\text{C}$ , or  $T_{m(k-1)} + 2.0^{\circ}\text{C} < \text{the MT between } T_{m(k-1)} \text{ and } T_{mk} < T_{mk} - 2.0^{\circ}\text{C}$ .

56. (withdrawn) The method of claim 46 wherein the MT between  $T_{mk}$  and  $T_{m(k+1)}$  is  $T_{mk} + 0.25^{\circ}\text{C} < \text{the MT between } T_{mk} \text{ and } T_{m(k+1)} < T_{m(k+1)} - 0.25^{\circ}\text{C}$ ,  $T_{mk} + 0.5^{\circ}\text{C} < \text{the MT between } T_{mk} \text{ and } T_{m(k+1)} < T_{m(k+1)} - 0.5^{\circ}\text{C}$ ,  $T_{mk} + 1.0^{\circ}\text{C} < \text{the MT between } T_{mk} \text{ and } T_{m(k+1)} < T_{m(k+1)} - 1.0^{\circ}\text{C}$ ,  $T_{mk} + 1.5^{\circ}\text{C} < \text{the MT between } T_{mk} \text{ and } T_{m(k+1)} < T_{m(k+1)} - 1.5^{\circ}\text{C}$ , or  $T_{mk} + 2.0^{\circ}\text{C} < \text{the MT between } T_{mk} \text{ and } T_{m(k+1)} < T_{m(k+1)} - 2.0^{\circ}\text{C}$ .

57. (withdrawn) The method of claim 46 wherein  $2 \leq n \leq 35$ ,  $2 \leq n \leq 18$ ,  $2 \leq n \leq 10$ ,  $2 \leq n \leq 7$ , or  $2 \leq n \leq 5$ .

58 (withdrawn) The method of claim 46 wherein the PCR mixture further comprises a FRET based probe.

59. (withdrawn) The method of claim 46 wherein the FRET based probe is selected from the group consisting of a Taqman probe, a double-dye oligonucleotide probe, an Eclipse probe, a Molecular Beacon probe, a Scorpion probe, a Hybridization probe, a ResonSense probe, a Light-up probe, and a Hy-Beacon probe.

60. (withdrawn) The method of claim 46 wherein the PCR mixture further comprises a second primer-based double stranded DNA dye that emits differently from the double stranded DNA dye.

61. (withdrawn) The method of claim 46 wherein the emission amount of the  $k$ th amplicon is obtained through a computer program performing the subtraction of the pre- $T_{mk}$  emission from the post- $T_{mk}$  emission or the subtraction of the post- $T_{mk}$  emission from the pre- $T_{mk}$  emission

62. (withdrawn) A computer software program for quantifying a real-time PCR amplicon which, when executed by a computer processor, performs the subtraction

of a pre- $T_m$  emission from a post- $T_m$  emission or the subtraction of the post- $T_m$  emission from the pre- $T_m$  emission.

63. (withdrawn) The computer software program of claim 62 wherein the emission was obtained from a double stranded DNA dye.

64. (withdrawn) The computer software program of claim 62 wherein the double stranded DNA dye is a double stranded DNA intercalating dye.

65. (withdrawn) The computer software program of claim 64 wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

66. (withdrawn) The computer software program of claim 62 wherein the double stranded DNA dye is a primer-based double stranded DNA dye that is covalently linked to the primers.

67. (withdrawn) The computer software program of claim 66 wherein the primer-based double stranded DNA dye is selected from the group consisting of fluorescein, FAM, JOE, HEX, TET, Alexa Fluor 594, ROX, and TAMRA, rhodamine, BODIPY-FI.

68. (withdrawn) The computer software program of claim 62 wherein a pre- $T_m$  emission is obtained at a MT below the  $T_m$  of the amplicon and a post- $T_m$  emission is obtained at a MT above the  $T_m$ .

69. (withdrawn) The computer software program of claim 68 wherein the MT below the  $T_m$  is 0.25 °C below, 0.5 °C below, 1.0 °C below, 1.5 °C below, or 2.0 °C below the  $T_m$ .

70. (withdrawn) The computer software program of claim 68 wherein the  $T_m$  is 0.25 °C above, 0.5 °C above, 1.0 °C above, 1.5 °C above, or 2.0 °C above the  $T_m$ .

71. (withdrawn) The computer software program of claim 62 which is stored and/or executed in a PCR instrument.

72. (withdrawn) The computer software program of claim 62 which is stored and/or executed in a computer connected to a PCR instrument.

73. (withdrawn) A computer program product comprising a computer memory having a computer software program, wherein the computer software program, when executed by a computer processor, performs the subtraction of a pre- $T_m$  emission from a post- $T_m$  emission or the subtraction of the post- $T_m$  emission from the pre- $T_m$  emission.

74. (withdrawn) The computer program product of claim 73 wherein the emission was obtained from a double stranded DNA dye.

75. (withdrawn) The computer program product of claim 73 wherein the double stranded DNA dye is a double stranded DNA intercalating dye.

76. (withdrawn) The computer program product of claim 75 wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

77. (withdrawn) The computer program product of claim 73 wherein the double stranded DNA dye is a primer-based double stranded DNA dye that is covalently linked to the primers.

78. (withdrawn) The computer program product of claim 77 wherein the primer-based double stranded DNA dye is selected from the group consisting of fluorescein, FAM, JOE, HEX, TET, Alexa Fluor 594, ROX, and TAMRA, rhodamine, BODIPY-FI.

79. (withdrawn) The computer program product of claim 73 wherein a pre- $T_m$  emission is obtained at a MT below the  $T_m$  of the amplicon and a post- $T_m$  emission is obtained at a MT above the  $T_m$ .

80. (withdrawn) The computer program product of claim 79 wherein the MT below the  $T_m$  is 0.25 °C below, 0.5 °C below, 1.0 °C below, 1.5 °C below, or 2.0 °C below the  $T_m$ .

81. (withdrawn) The computer program product of claim 79 wherein the MT above the  $T_m$  is 0.25 °C above, 0.5 °C above, 1.0 °C above, 1.5 °C above, or 2.0 °C above the  $T_m$ .

82. (withdrawn) The computer program product of claim 73 which is stored and/or executed in a PCR instrument.

83. (withdrawn) The computer program product of claim 73 which is stored and/or executed in a computer connected to a PCR instrument.

84. (withdrawn) A PCR instrument comprising the computer program product of claim 73.